Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Molecular Aspects of Sex Determination in Mice: An Alternative Model for the Origin of the Sxr Region [and Discussion]

C. E. Bishop, A. Weith, M.-G. Mattei, C. Roberts and B. M. Cattanach

Phil. Trans. R. Soc. Lond. B 1988 **322**, 119-124
doi: 10.1098/rstb.1988.0119

References Article cited in:
http://rstb.royalsocietypublishing.org/content/322/1208/119#related-urls

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1988 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Phil. Trans. R. Soc. Lond. B **322**, 119–124 (1988) [119]
Printed in Great Britain

Molecular aspects of sex determination in mice: an alternative model for the origin of the Sxr region

By C. E. Bishop[1], A. Weith[1], M.-G. Mattei[2] and C. Roberts[1]

[1]*Departement d'Immunologie, Unité d'Immunogénétique humaine, INSERM U-276, Institut Pasteur, 25 Rue du Dr Roux, Paris, France*

[2]*Groupe Hospitalier de la Timone, INSERM U-242, 13385 Marseille, Cedex 5, France*

Using a combination of *in situ* mapping and DNA analysis with recombinant DNA probes specific for the Sxr region of the mouse Y chromosome, we show that both the gene(s) controlling primary sex determination and the expression of the male-specific antigen H-Y (*Tdy* and *Hya* respectively) are located on the minute short arm of the mouse Y chromosome. We demonstrate that the H-Y$^-$ variant of Sxr (Sxr′) arose by a partial deletion within the Sxr region and propose an alternative model for the generation of the original Sxr region.

Introduction

Sex reversed (Sxr, Tp(Y)1Ct) is a small fragment of the mouse Y chromosome which has transposed to the distal pairing–recombination region (the pseudoautosomal region) of the Y in XYSxr mutant mice (Singh & Jones 1982; Evans *et al.* 1982; Hansmann 1982; Eicher & Washburn 1986). During male meiosis this fragment is regularly transferred by recombination to the paternal X, giving rise to sterile XXSxr (sex-reversed) males. It has been shown that Sxr contains the genes controlling primary sex-determination (*Tdy*) and the H-Y transplantation antigen (*Hya*) as defined by graft rejection and by the cytotoxic T-cell assay (Simpson *et al.* 1984). An H-Y negative variant (Sxr′) has also been described (McLaren *et al.* 1984) and it has been suggested that H-Y expression may play a role in spermatogenesis (Burgoyne *et al.* 1986). Sxr contains a high concentration of simple Bkm-related repeated sequences that, when used to probe the YSxr chromosome *in situ*, show a heavy concentration of grains in the pericentric region and another peak around the telomere (Singh & Jones 1982). This led to the conclusion that Sxr represented a fragment transposed from the subcentromeric region of the Y distal to the pseudoautosomal region. Hence *Tdy* and *Hya* were mapped to a region just below the centromere of the normal Y. We present here direct cytological and molecular data showing that Sxr is, in fact, derived from the normal Y-chromosome short arm.

Analysis of the Sxr region using DNA probes

Recently, we have isolated random DNA probes recognizing sequences within the Sxr region of the mouse and have used them to analyse this mutation at the molecular level (Bishop *et al.* 1987). One probe, pYCR8, is a 2Kb *Eco*RI fragment isolated from a Y-chromosome-enriched library. Figure 1*a* shows that, on genomic blots of *Taq*-1-restricted DNA, pYCR8 detects four bands in the XY and XYSxr males but fails to react with female DNA, showing them to be Y-located. All bands can be found in DNA from the XXSxr male, demonstrating that they are located within the Sxr region. In an analysis of over 100 backcross mice, co-segregation of Sxr

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* STOR www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

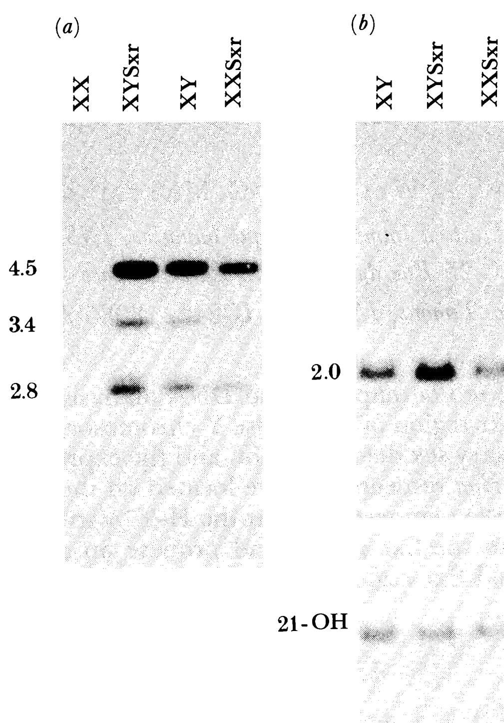


FIGURE 1. (*a*) Southern blot analysis of *Taq*1-restricted DNA from an XX female, XYSxr male, XY male and an XXSxr male by using pYCR8. (*b*) Southern analysis of the same DNAs as in (*a*) restricted by *Eco*RI. Lower panel shows the result of reprobing the same blot with the autosomal 21-OH probe to quantify the amount of DNA present. Methods: high molecular mass DNA was extracted from single mouse livers by standard procedures, 15 μg was digested with either *Taq*1 or *Eco*RI, separated on 0.8% agarose gels transferred to Hybond-N membranes (Amersham) and fixed by UV irradiation. Membranes were hybridized to [^{32}P]pYCR8 by random priming (Feinberg & Volgelstein 1984) (specific activity $> 5 \times 10^8$ counts min^{-1} μg^{-1}). The blots were then washed stringently (0.1 × SSC†, 68 °C) and exposed moist to XAR-5 film (Kodak) with an intensifying screen at −80 °C for 18–24 h.

and pYCR8 was always observed (Nagamine *et al.* 1987). In addition, with Alu1- or HaeIII-digested DNA the probe did not hybridize to the Sxr-specific high molecular mass bands identified by Bkm, showing it to lie outside these sequences (data not shown). Figure 1*b* shows the result of probing *Eco*RI-digested DNA from XY, XYSxr and XXSxr males. pYCR8 detects the 2 kilobase cognate sequence and in addition two homologous bands of 2.6 and 2.8 kilobases, both of which map to the Sxr region. Densitometric scanning of the autoradiographs revealed that all three bands were twice as intense in the XYSxr carrier male as in the normal XY male or the XXSxr male. The amount of DNA in each lane was quantitated by reprobing with a steroid 21-hydroxylase probe (located on chromosome 17). Further dosage analysis showed the cognate band to be present as a single copy in normal male C57BL/6 XY DNA (not shown). Because of the unique nature of this probe we were able to use it to define accurately the region of the Y that has been duplicated in the YSxr chromosome. Figure 2 shows the results of *in situ* hybridization of pYCR8 to mouse metaphase chromosomes of a normal C57BL/6 male. Contrary to expectations the peak of hybridization on the normal Y was not underneath the centromere but above it. The silver grains in fact define the short arm of the Y. Although the

† 1 × SSC = 0.15 M sodium chloride + 0.015 M sodium citrate.

Downloaded from rstb.royalsocietypublishing.org

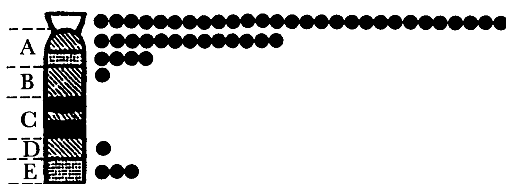


FIGURE 2. Hybridization *in situ* of probe pYCR8 to the Y chromosomes of a C57BL/6 normal male. Methods: the entire pYCR8 plasmid was tritium-labelled by nick translation to a specific activity of approximately 0.7×10^8 disintegrations min^{-1} μg^{-1}. Concanavalin-A-stimulated lymphocytes from C57BL/6, WMP and XYSxr male mice were cultured at 37 °C for 72 h and 5-bromodeoxyuridine added for the final 5.5 h of culture to ensure good post-hybridization chromosomal R-banding and optimal visualization of the Y short arm. Metaphase cells were hybridized by using a probe concentration of 10 ng ml^{-1} in the hybridization mixture as previously described (Dautigny *et al.* 1986). Slides were covered with kodak NTB2 nuclear track emulsion and exposed for 15 days at 4 °C. After development the chromosome spreads were first stained with buffered Giemsa solution and the metaphases photographed. R banding was then done by the fluorochrome-photolysis-Giemsa method and the metaphases rephotographed before analysis. More than 100 metaphases were examined.

existence of a Y short arm is not well documented, it was first described by Ford (1966) and is frequently used to identify the Y in cytogenetic studies (P. Burgoyne, E. Evans & E. Eicher, personal communications). This immediately suggests a simpler explanation for the origin of Sxr involving the relocation of the entire (or a portion) of the Y short arm containing *Tdy* and *Hya* to the pseudoautosomal region. This has the advantage of involving only one breakpoint and the mobilization of the short arm telomere.

With this model in mind we investigated the generation of the H-Y negative XXSxr′ mouse (McLaren *et al.* 1984). Probing the DNA of this mouse with pYCR8 revealed no differences in the hybridization profiles. Figure 3, however, shows the result of probing *Bam*HI-digested DNA from an XY male, XX female (C57BL/6 strain), XXSxr, XXSxr′ (latter two from an N9 generation backcrossed onto C57BL/6) with the probe pY291. This probe is a 2.6 kilobase *Eco*RI fragment taken from a Y chromosome microcloned library (A. Weith & C. E. Bishop, unpublished data). Four Y-located bands are detected in the normal XY male at approximately 12.0 kilobases (band A), 9.0 kilobases (band B), 6.0 kilobases (band C) and 3.0 kilobases (band D). These bands are not present in female DNA. All four are present in XXSxr although 291B and 291C are reduced in intensity. This suggests that 291B and C are repeated on the Y but only a limited fraction of them map within Sxr. A comparison of the banding pattern of XXSxr with XXSxr′ reveals a deletion of band 291A whereas the other bands remain unchanged. These data clearly show that the generation of the Sxr′ ($H\text{-}Y^-$) mouse from Sxr ($H\text{-}Y^+$) was not merely because of a point mutation in *Hya* (leading to loss of H-Y expression) but involved a partial deletion event within Sxr itself.

Based on these data we propose the following model for the origin of Sxr and Sxr′ (see figure 4):

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

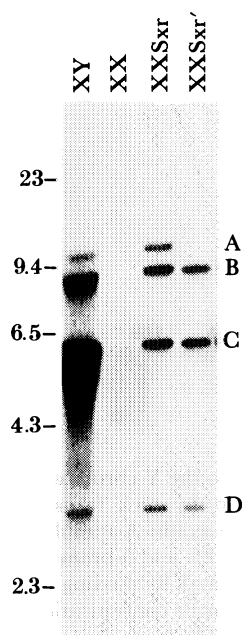


Figure 3. Southern blot analysis of *Bam*HI digested DNA from XY male (CB′ strain), XX female (C57BL/6 strain), XXSxr and XXSxr′ males with pY291. With the exception of the XX female all mice were kindly provided by Anne McLaren.

the Sxr region containing the genes (structural or controlling) for *Tdy* and *Hya* maps to the short arm of the mouse Y chromosome (figure 4*a*). Sxr was generated by the relocation of the whole or part of this arm from one chromatid to the distal pairing region of the sister chromatid by transposition or non-homologous exchange. Hence only one chromosomal break need be postulated and the short-arm telomere would then become the telomere of the YSxr chromosome long arm. At the DNA level this Sxr fragment would contain Sxr-specific sequences recognized by pYCR8 and pY291 (represented by an □ and ○ respectively in figure 4), a concentration of simple repeats (GATA/GACA) and several Y-specific (but not Sxr-specific) repeats recognized by pY291 (represented by a closed circle in figure 4). The placing of the ancestral Sxr region on the short arm of the Y is not in conflict with the results of Bkm hybridization *in situ*, as this latter probe is too highly repeated on the Y to resolve the short arm. The generation of Sxr′ from Sxr was a relatively simple event bearing in mind that the original father was carrying Sxr on both the X and the Y chromosomes (XSxr/YSxr). An unequal recombination event could have occurred between the two Sxr regions during male meiosis, leading to the deletion of DNA carrying all or part of the H-Y gene *Hya* (figure 4*b*). At the DNA level this would involve the loss of Sxr-specific unique sequences recognized by pY291. Consistent with this model are the surprisingly high rates of unequal crossover recently reported by Harbers *et al.* (1986) for the mouse pseudoautosomal region.

Finally, the detection of this DNA deletion within the Sxr region correlated with the loss of H-Y antigen expression may open the way to the molecular cloning of *Hya* itself (or its controlling gene) and allow one to test the hypothesis that it is involved in spermatogenesis. To this end we are at present constructing a long-range map of the Sxr region by using pulsed-field

Downloaded from rstb.royalsocietypublishing.org

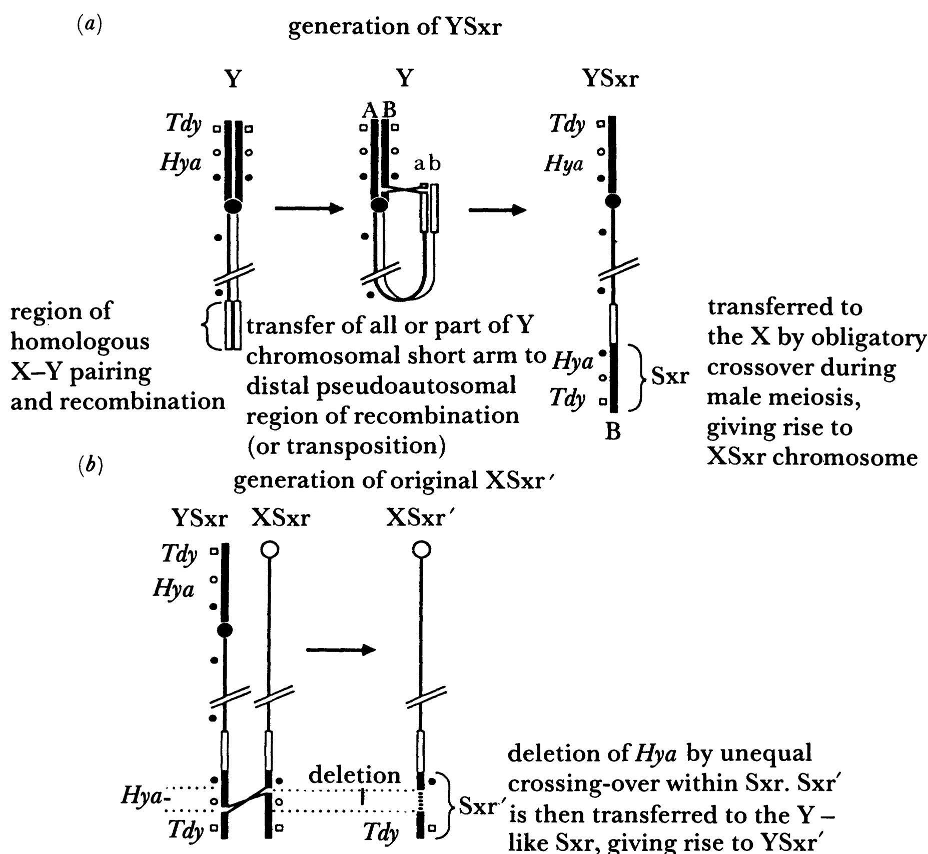


FIGURE 4. Proposed origin of Sxr and Sxr′. Open box (Â) represents the unique Sxr-specific sequence recognized by pYCR8. (○) The unique sequence detected by pY291 which is deleted in Sxr′; (●) the repeated sequences detected with this probe. The exact location of these sequences relative to each other has yet to be determined. Similarly the order of *Tdy* and *Hya* is arbitrary.

gel electrophoresis and multiple Sxr-specific DNA probes. This should allow a minimum size estimate of the deletion to be made as a first step to cloning the Y-located gene.

We acknowledge the continuous interest and support of Dr M. Fellous, Dr P. Avner, Dr C. Rijnders and Dr D. Traud during the course of this work, J. Weissenbach for critical comments, J.-L. Guenet for the WMP strain and Anne McLaren, F.R.S., for the Sxr′ mice. We acknowledge the financial support of the Fondation pour la Recherche Médicale (C.R.), the Deutsche Forschungsgemeinschaft (A.W.) and INSERM.

REFERENCES

Bishop, C. E., Roberts, C., Michot, J.-L., Nagamine, C., Winking, H., Guenet J.-L. & Weith, A. 1987 The use of specific DNA probes to analyse the Sxr mutation in the mouse. *Development* **101** (suppl.), 167–175.

Bishop, C. E. & Hatat, D. 1987 Molecular cloning and sequence analysis of a mouse Y chromosome transcript expressed in the testis. *Nucl. Acids Res.* **15**, 2959–2969.

Burgoyne, P., Levy, E. & McLaren, A. 1986 Spermatogenic failure in male mice lacking H-Y antigen. *Nature, Lond.* **320**, 170–172.

Dautigny, A., Mattei, M.-G., Morrello, D., Alliel, P. M., Pham-Dinh, D., Amar, L., Arnaud, D., Simon, D., Mattei, J.-F., Guenet, J.-L., Jolles, P. & Anver, P. 1986 The structural gene coding for the myelin-associated proteolipid protein is mutated in *jimpy* mice. *Nature, Lond.* **321**, 867–869.

Downloaded from rstb.royalsocietypublishing.org

Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. 1982 Meiotic crossing over between the X and Y chromosomes of male mice carrying the sex-reversing factor (Sxr). *Nature, Lond.* **300**, 443–445.
Eicher, E. M. & Washburn, L. L. 1986 Genetic control of primary sex determination in mice. *A. Rev. Genet.* **20**, 327–360.
Feinberg, A. P. & Vogelstein, B. 1984 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analyt. Biochem.* **137**, 266–267.
Ford, C. E. 1966 Communication. *Transplantation* **4**, 333–335.
Hansmann, I. 1982 Sex reversal in the mouse. *Cell* **30**, 331–332.
Harbers K., Soriano, P., Muller, U. & Jaenisch, R. 1986 High frequency of unequal recombination in pseudoautosomal region shown by proviral insertion in transgenic mice. *Nature, Lond.* **324**, 682–685.
McLaren, A., Simpson, E., Tomonari, K., Chandler, P. & Hogg, H. 1984 Male sexual differentiation in mice lacking H-Y antigen. *Nature, Lond.* **312**, 552–555.
Nagamine, C. M., Michot, J.-L., Roberts C., Guenet, J.-L. & Bishop, C. E. 1987 Linkage of the murine steroid sulfatase locus, *Sts*, to sex reversed, *Sxr*,: a genetic and molecular analysis. *Nucl. Acids Res.* **15**, 9227–9238.
Simpson, E., McLaren, A., Chandler, P. & Tomonari, K. 1984 Expression of H-Y antigen by female mice carrying Sxr. *Transplantation* **37**, 17–22.
Singh, L. & Jones, K. W. 1982 Sex reversal in the mouse is caused by a recurrent non-reciprocal cross-over involving the X and an aberrant Y chromosome. *Cell* **28**, 205–216.

Discussion

B. M. Cattanach (*M.R.C. Radiobiology Unit, Didcot, U.K.*). The segregation of Sxr appears to be that expected when pooled data from many XY Sxr males is considered. However, individual XY Sxr males have occasionally been observed to produce abnormal segregation ratios. When carried on the X, rather than the Y, an abnormal segregation has very clearly been seen recently. Thus such males have been found to produce very few XX daughters, an excess of XX Sxr sons, few XY Sxr sons and excess XY non-Sxr sons.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

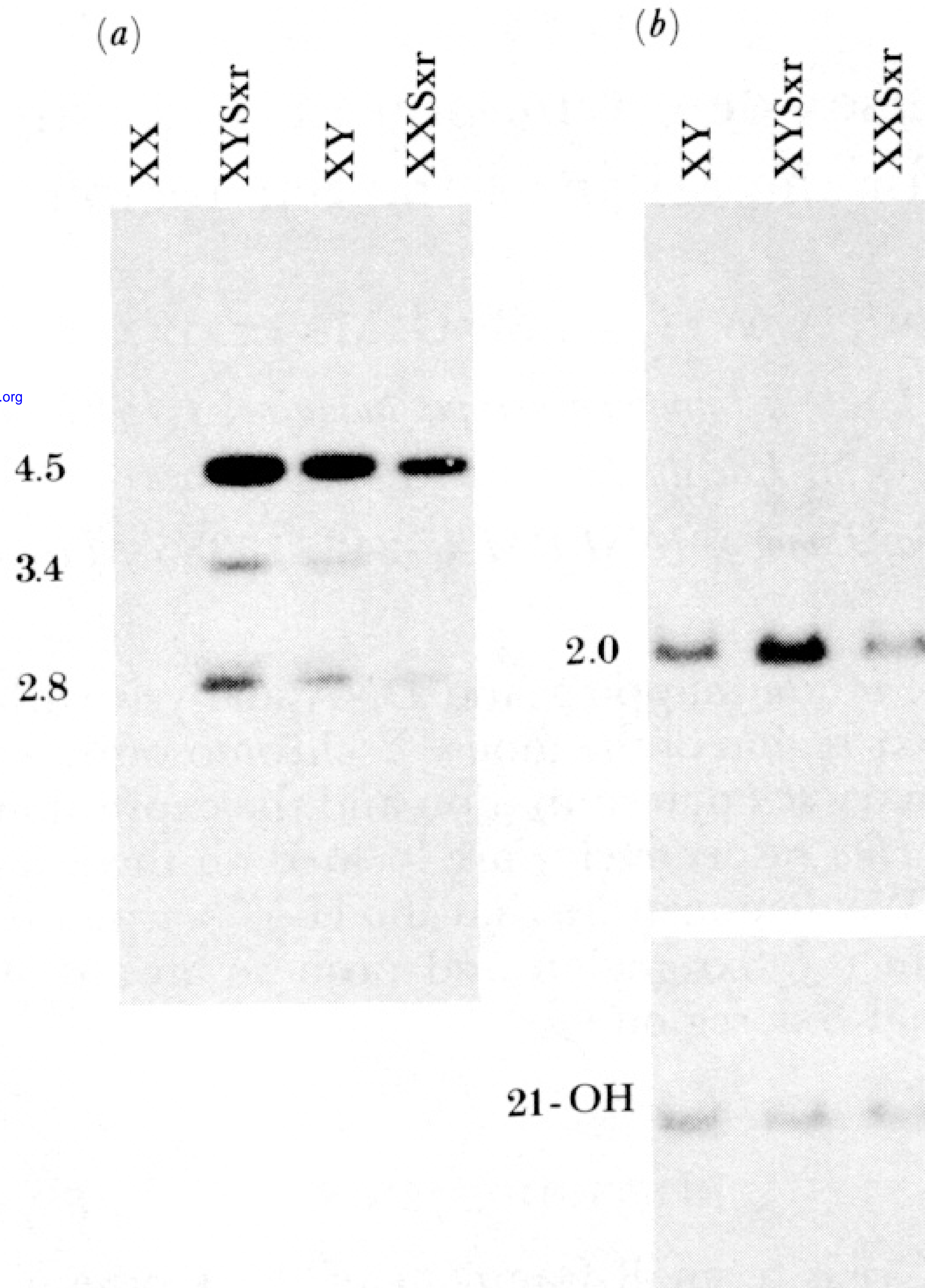
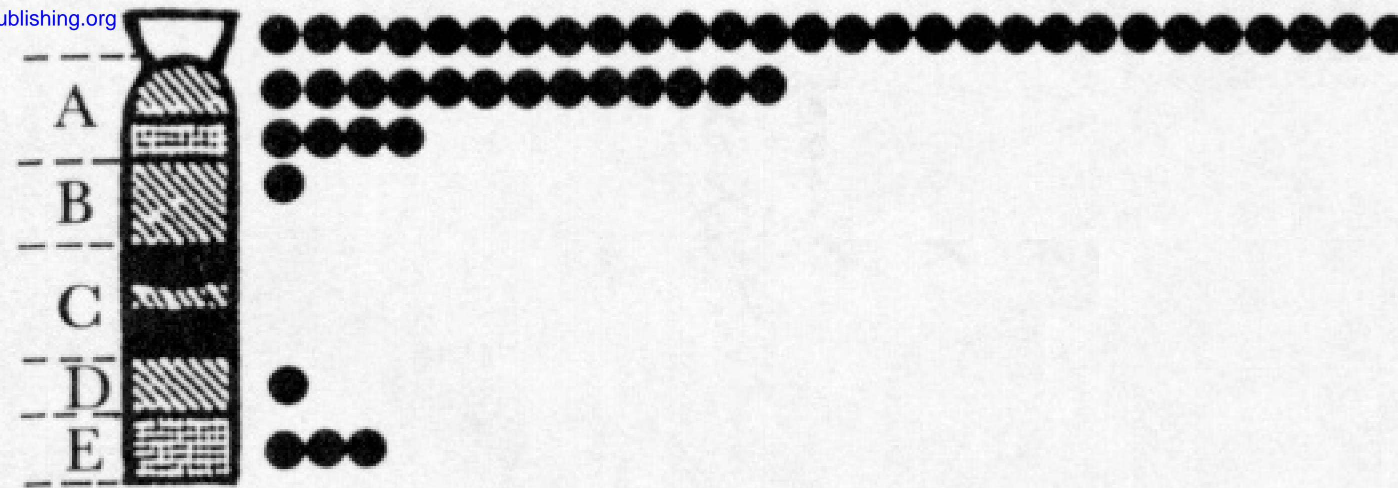


FIGURE 1. (*a*) Southern blot analysis of *Taq*1-restricted DNA from an XX female, XYSxr male, XY male and an XXSxr male by using pYCR8. (*b*) Southern analysis of the same DNAs as in (*a*) restricted by *Eco*RI. Lower panel shows the result of reprobing the same blot with the autosomal 21-OH probe to quantify the amount of DNA present. Methods: high molecular mass DNA was extracted from single mouse livers by standard procedures, 15 μg was digested with either *Taq*1 or *Eco*RI, separated on 0.8% agarose gels transferred to Hybond-N membranes (Amersham) and fixed by UV irradiation. Membranes were hybridized to [^{32}P]pYCR8 by random priming (Feinberg & Volgelstein 1984) (specific activity > 5×10^8 counts min^{-1} μg^{-1}). The blots were then washed stringently (0.1 × SSC†, 68 °C) and exposed moist to XAR-5 film (Kodak) with an intensifying screen at −80 °C for 18–24 h.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



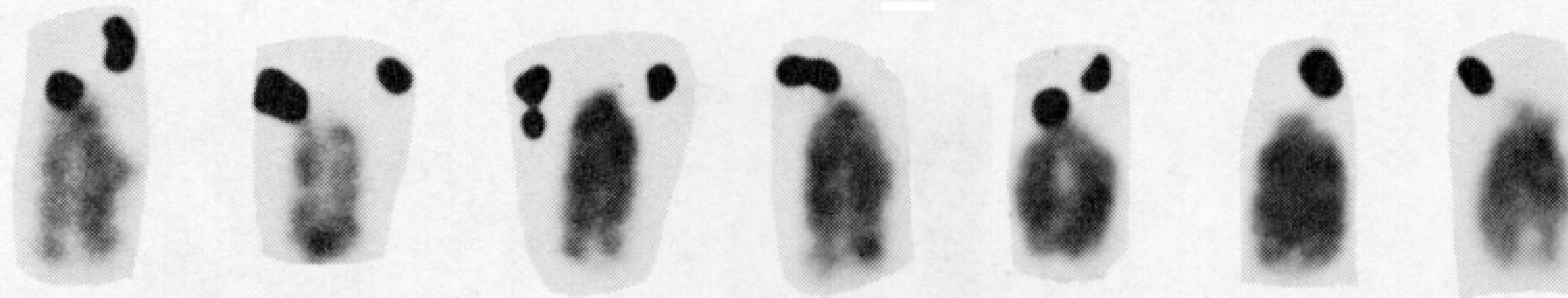


Figure 2. Hybridization *in situ* of probe pYCR8 to the Y chromosomes of a C57BL/6 normal male. Methods: the entire pYCR8 plasmid was tritium-labelled by nick translation to a specific activity of approximately 0.7×10^8 disintegrations min^{-1} μg^{-1}. Concanavalin-A-stimulated lymphocytes from C57BL/6, WMP and XYSxr male mice were cultured at 37 °C for 72 h and 5-bromodeoxyuridine added for the final 5.5 h of culture to ensure good post-hybridization chromosomal R-banding and optimal visualization of the Y short arm. Metaphase cells were hybridized by using a probe concentration of 10 ng ml^{-1} in the hybridization mixture as previously described (Dautigny *et al*. 1986). Slides were covered with kodak NTB2 nuclear track emulsion and exposed for 15 days at 4 °C. After development the chromosome spreads were first stained with buffered Giemsa solution and the metaphases photographed. R banding was then done by the fluorochrome-photolysis-Giemsa method and the metaphases rephotographed before analysis. More than 100 metaphases were examined.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

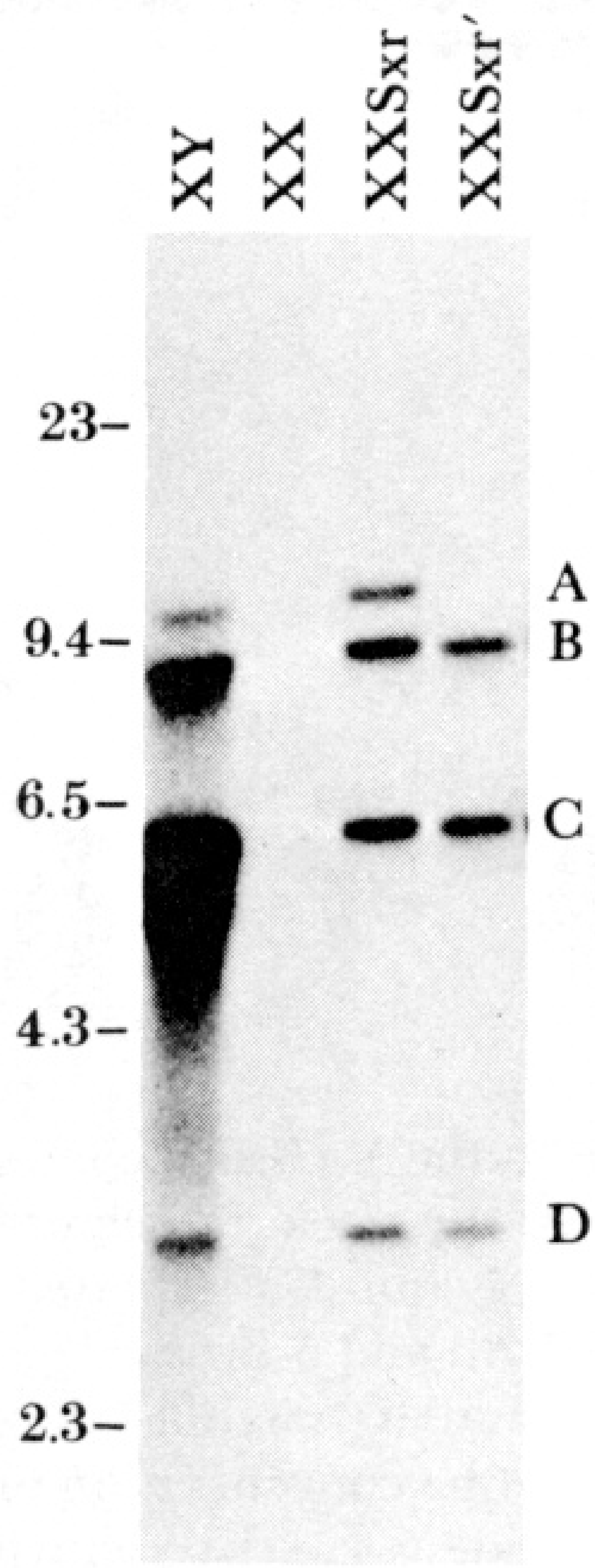


FIGURE 3. Southern blot analysis of *Bam*HI digested DNA from XY male (CB′ strain), XX female (C57BL/6 strain), XXSxr and XXSxr′ males with pY291. With the exception of the XX female all mice were kindly provided by Anne McLaren.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES